Proceedings of the First Freshwater Mollusk Conservation Society Symposium, 1999, pages 203-218

How much excavation is needed to monitor freshwater mussels?

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ABSTRACT: To examine variation in detecting freshwater mussels at the substrate surface, we counted mussels on and below the substrate surface by excavating 907 0.25 m^2 quadrats to a depth of 10 cm at wadeable sites in 14 streams in 9 states. Probability of detection on the surface was related to water depth, coverage of rooted vegetation, substrate type, and mussel species and length (χ^2 = 174.3, df=25, P<0.0001). These factors interacted to cause large differences in the probability of detection among habitat types and species. As a result, when detection differed among sites for the same mussel species (e.g., percent at the surface of *Alasmidonta heterodon* was 22% at one site and 64% at another), surface density was an unreliable surrogate for comparing true density. When detection differed markedly among species within a site (*e.g.*, at French Creek, 25% of *Villosa fabalis* was detected at the surface compared to 71% of *Actinonaias ligamentina*), surface counts did not accurately measure relative abundance. Our results indicate that some amount of excavation is necessary for rigorous comparison of density across sites, time, habitat, or taxa. We considered application of the double sampling design to weigh the costs and benefits of excavation and determine the proportion of quadrats to excavate that minimizes variance of population estimates for a fixed cost of sampling. We found that the optimal proportion to excavate depends on the percent of mussels detected at the substrate surface. If >60% are likely to be detected at the surface then excavation of 25% (or 1 of 4) of the quadrats will minimize variance. Similarly, 50 - 60% detection at the surface leads to excavating 33% of the quadrats; 40 - 50% detection at the surface leads to excavating 50%; and <40% detection at the surface leads to excavating 100%. The double sample design could be useful for monitoring low-density populations. For example, at a site where mussel density is 0.2 m^{-2}, sample size of 400 would result in an estimate with CV of 0.36 and power ≥0.80 to detect declines to 0.02 m^{-2} over 5 y or 0.07 m^{-2} over 10 y.

Keywords: freshwater mussels, population assessment, monitoring, sampling design, excavation, costs and benefits, precision, uncertainty, statistical power, sample size

Excavation is the process of removing and sifting through stream substrates to collect and count mussels, and it is one possible technique in a protocol for sampling freshwater mussel populations (Miller and Payne 1988). Biologists use excavation because not all mussels are detected at the substrate surface, and because detection at the surface can change across sites or with season. If detection changes from one site (or season) to another then comparisons based on counts of mussels at the surface will not provide an accurate comparison of population density.

Biologists have expressed differing opinions on the need for excavation in mussel surveys. Miller and Payne (1988) equate excavation with accurate or "quantitative" sampling, and use the term "semi-quantitative" for counting mussels by tactile searches of the substrate surface. They state that tactile searches underestimate densities of smaller individuals and should be limited to assessments of distribution or relative abundance. In contrast, Strayer and Ralley (1993) did not excavate in their study of habitat use. They based their analysis on visual searches for mussels at the substrate surface, although they did lift non-embedded stones to find mussels.

Several recent studies specifically addressed distribution of mussels at and below the substrate surface (*i.e.*, vertical distribution). Amyot and Downing (1991) examined vertical distribution of *Elliptio complanata* (Rafinesque, 1820) in a sand-bottomed lake in Québec, Canada, and reported that the proportion of mussels found at the substrate surface varied seasonally. Percent at the surface peaked in early summer (*i.e.*, >96%) and declined in the autumn months (*i.e.*, < 40%). They also found that the percent below the substrate surface was related to mussel length with smaller mussels being more likely to be buried. Balfour and Smock (1995) studied populations of *E. complanata* in a sand-bottomed stream in Virginia, USA. Their results were qualitatively similar to those of Amyot and Downing (1991); they found significant seasonal and length-related variation in the proportion of mussels found at the substrate surface. Richardson and Yokley (1996) surveyed sites on the Apalachicola River, Florida, USA for evidence that *Amblema neislerii* (Lea, 1858) or *Glebula rotunda* (Lamarck, 1819) had experienced

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recent recruitment. Previous surveyors of these sites applied only visual or tactile searches of the substrate surface and failed to find evidence of reproduction or recruitment. However, Richardson and Yokley (1996), who included excavation in their survey, found juveniles and concluded that excavation is necessary to assess recruitment.

Excavation, however, has its costs. Mussels and their habitat are disturbed when substrate is removed and sifted. Although we found no documentation in the literature, we hypothesized that excavation could cause an increase in mortality, especially for small, thin-shelled, or juvenile mussels. It is also possible that excavation interferes with reproduction, an effect that would most likely occur if the survey coincided with periods of reproductive activity.

Relative to surface counts excavation is time-consuming. The amount of time available to conduct a survey is always limited. More quadrats could be sampled (and more of the site covered) if excavation is not applied. Thus, more excavation means less spatial coverage. Because mussels tend to cluster, a sample with less spatial coverage results in a population estimate that is less precise especially at low population densities.

We considered the application of the double sampling design (Thompson 1992) to minimize the amount of excavation required to achieve accurate and precise population estimates. Typically, the design stipulates that samples are taken in two phases. During the first phase, an inexpensive (but inaccurate) sampling method is applied to a large, random sample. Subsequently, during the second phase, an expensive (but more accurate) sampling method is applied to a random subset of the first-phase sample. The second-phase sample is used to model the relationship between the two methods, and then the model is used to calibrate the response for the remainder of the first-phase sample.

Our objectives were to: 1) examine variation in detection at the surface, 2) evaluate the application of double sampling to sampling freshwater mussels, 3) determine the amount of excavation that would minimize variance of population estimates for fixed cost, and 4) calculate sample size required to achieve desired levels of precision and power to detect population change. We used a case study approach to identify factors that affect detection and used data from the case study to evaluate the application of double sampling. After determining the optimal amount of excavation, we conducted a series of sample size calculations to examine the magnitude of population change likely to be detected when monitoring mussels over multiple years.

Table 1. Locations of the 14 sites surveyed during June-September 1997 and the number of 0.25 m^2 quadrats that were excavated in wadeable water.

Major drainage	Site	State	Watershed	Excavated quadrats [a]
Atlantic Slope	Ashuelot River	NH	Connecticut River	65
	Cacapon River	WV	Potomac River	61
	Connecticut River	NH/VT	Connecticut River	49
	Little River	NC	Neuse River	41
	Neversink River	NY	Delaware River	62
	Norwich Creek	MD	Choptank River	40
	Piscataquog River	NH	Merrimack River	100
	St. George River	ME	St. George River	99
	West River	VT	Connecticut River	69
	Farmington River (W. Br.)	MA	Connecticut River	50
Interior Basin	Allegheny River	PA	Allegheny River	118
	French Creek	PA	Allegheny River	28
	Little Tennessee	NC	Tennessee River	24
St. Lawrence River	Poultney River	NY/VT	Lake Champlain	101

[a] In addition, surface counts alone were conducted on an approximately equal number of quadrats.

Methods

Factors affecting detection: a case study

During June through September 1997, we surveyed sites in 14 streams: 10 systems were in the Atlantic Slope, 3 were Interior Basin Drainages, and 1 was in the St. Lawrence River drainage (Table 1). At each site 0.25 m^2 quadrats were systematically placed. Positions along a bank were selected at equal intervals after a random start, and quadrats were placed at equal intervals across the stream after a random start from each bank position. Mussels at the substrate surface were collected, counted, identified to species, measured along their longest axis, and re-embedded in the substrate. Searches at the substrate surface were conducted while snorkeling, or through a glass-bottomed bucket, in wadeable water (<1.5 m). Observation was visual or tactile depending on turbidity. As part of the search, fine sediment was fanned away, non-embedded material was lifted and loose sediment was raked with fingertips in an effort to detect mussels at the surface. We excavated every other quadrat (50% of all quadrats) after the surface count was completed. Excavation consisted of removal of substrate to a depth of approximately 10 cm and sifting substrate through a mesh screen with openings of 6.4 mm. Altogether 907 quadrats were excavated at the 14 sites in wadeable waters. We recorded time to complete the surface count and excavation separately.

To test the hypothesis that excavation increases mortality of mussels when compared to surface counts, we placed mussels in plastic cages that resembled "milk crates" (30.5 cm x 35.6 cm x 25.4 cm) in the stream for at least 7 wks at 3 sites. We monitored mussel survival as a function of removal during a surface count or during excavation. The cages contained sediment, were wrapped in plastic mesh screen with openings of 3.2 mm, and were anchored to the stream bottom.

We recorded turbidity (LaMotte model 2008 turbidity meter) and temperature for each day of sampling. At each quadrat we recorded the observer, macrohabitat (riffle, run, pool), substrate size using the Wentworth scale (Gordon *et al*. 1992), depth, and percent rooted vegetation. Because the same observers did not visit all sites nor survey all habitat types within a site, observer effects could have been confounded by habitat effects. We used logistic regression (see below) to test whether detection differed among observers within each site prior to modeling detection across sites. For those sites where an observer effect was apparent we conditioned the test on habitat. Because sample sizes were often small (*i.e.*, expected values <5 for >20% of observer, habitat combinations), we used exact methods for these latter tests (Mehta and Hilton 1993).

We used logistic regression (Hosmer and Lemeshow 1989) to model and test whether the probability of detection at the substrate surface was related to habitat, observer, or mussel length. In this analysis the response variable was whether a mussel was detected at the surface or not, and habitat, observer, and mussel length were the explanatory variables. We compared models using likelihood ratio statistics to test for effects of explanatory variables and their interactions. Akaike's Information Criteria (AIC; Burnham and Anderson 1998) was used to select the model which best explained detection at the surface. Extra-binomial variation was accounted for by William's method (Williams 1982). To assess adequacy of the model and look for systematic lack of fit we plotted a series of diagnostic statistics (Hosmer and Lemeshow 1989).

We determined the correct scale for the relationship between response and explanatory variables by plotting the response on the logit scale against each explanatory variable (Hosmer and Lemeshow 1989). This procedure provided some evidence of nonlinearity for depth and length, thus quadratic terms were added to the model for these variables. Although cubic terms were included, model fit was not improved so we present results only for models including quadratic terms. To aid interpretation of interactions, we converted depth and percent vegetation from continuous to ordinal. The categories for depth were <0.25 m, 0.25 to 0.75 m, and 0.75 to 1.5 m. The categories for percent rooted vegetation were 0%, 0 to 33%, and >33%.

To assess the usefulness of surface counts for determining relative abundance, we ranked species density within each site using both surface and total counts and looked for discrepancies between the two lists of relative abundance. When the lists of ranks differed we tested for statistical significance by ordering the species according to ranked total counts and testing for a shift in ranks using the Wilcoxon test.

The optimal proportion of quadrats to excavate

We determined the optimal amount of excavation in the context of a double sampling design (Thompson 1992). In the first phase of the double sampling design, mussels are counted on the surface in a large random sample of quadrats. In the second phase, a representative subset of the first-phase sample is selected, and these quadrats are excavated. The second-phase sample is sometimes referred to as a calibration sample (Luo *et al*. 1998).

Surface counts and total counts (total count = count below the surface + count at the surface) from the calibration sample are used to calibrate the surface counts for the entire sample.

The ratio estimator and the regression estimator are two common estimators available under the double sampling design (Thompson 1992). In the estimators, the total count is the response variable (y) and the surface count is the explanatory variable (x). The ratio estimator is based on the assumption that if x=0, then y=0. However, the regression estimator does not require that assumption; it allows x=0, but y>0. Thus, we recommend the regression estimator because it allows for the probable event that mussels are found during excavation even though none are detected on the surface. A regression model (*e.g.*, a simple linear regression model) is fit to the data from the second-phase sample. We present formulae for the regression estimator under double sampling in the Appendix.

To determine the optimal proportion of quadrats to excavate, we found the proportion that minimized variance of the population density estimate for a fixed total cost. We considered 3 costs: time to set up and move around the site (c*), time to count mussels on the surface of a quadrat (c'), and time to excavate a quadrat (c). We set total cost to be $C = c^* + c'n' + cn$, where n' was the sample size for the first phase of sampling, and n was the sample size for the second phase. If C is fixed, then variance of the population estimate is a function of the proportion of the first-phase sample that is excavated (*i.e.*, the "proportion to excavate" or n/n'). We wanted to find the proportion that resulted in the smallest variance given that total cost was fixed. We found this "optimal proportion to excavate" by Thompson (1992)

$$\tilde{f}_2 = \frac{n}{n'} = \sqrt{\frac{c'}{c}\left[\frac{s^2}{s_{lr}^2} - 1\right]^{-1}}, \qquad \textbf{(1)}$$

where s^2 was the variance in total counts among excavated quadrats and s_{lr}^2 was the mean square error from the regression between surface and total counts. Using the relationship between s^2, s_{lr}^2, and an adjusted version of R^2 (Ryan 1997), we wrote an equivalent formula for the optimal proportion to excavate as

$$\tilde{f}_2 = \frac{n}{n'} = \sqrt{\frac{c'}{c}\left[\frac{1 - R^2_{adjusted}}{R^2_{adjusted}}\right]}. \qquad \textbf{(2)}$$

We used Eqn. 1 to calculate the optimal proportion to excavate for the 14 sites in our study. We combined all species at a site for these analyses. For each site we fit a simple linear regression of total count against surface count and computed average times to complete a surface count (c') and an excavation (c).

We calculated variance and coefficient of variation (CV) for a range of sample sizes (*i.e.*, sample size = the total number of quadrats in the first phase of sampling = n'). We made the simplifying assumption that n' was a negligible fraction of the possible number of quadrats at a site (N), which is a reasonable assumption for mussel surveys where area sampled tends to be <5% of the area at the site. This is a conservative assumption in that our sample size calculations will overestimate sample size needed to achieve a desired precision especially for small sites (*e.g.*, <1000 m^2). From this assumption we derived a simplified version of the variance

$$\mathrm{var}\left(\hat{\mu}\right) \cong \frac{s^2}{a^2 n'}\left(R^2_{adjusted} + \frac{1 - R^2_{adjusted}}{\tilde{f}_2}\right), \qquad \textbf{(3)}$$

where $\tilde{f}_2$ is the optimal proportion to excavate, which we found using Eqn. 1. In addition to using Eqn. 1 to calculate the optimal proportion to excavate, we examined the functional form of the relationship between variance and proportion excavated by plotting the variance from Eqn. 3 for $R^2_{adjusted}$ of 0.4, 0.6, and 0.8. For these latter calculations the $s^2/(a^2 n')$ term in Eqn. 3 was constant and did not affect the form of the variance curve.

We conducted a power analysis to determine how sensitive the survey design was to changes in density if the survey were to be repeated annually for 5 or 10 y (or biannually for 10 or 20 y, for example). We used the program TRENDS (Gerrodette 1987, Thompson *et al*. 1998) to calculate the minimum change in density that would be detected in surveys of 5 and 10 y with probability > 0.80 for 1 tailed t-tests where α= 0.10. To generalize the power analysis, we determined the relationship between CV and density given sample size. For a range of densities and sample sizes we used the relationship to predict CV, which was then entered into program TRENDS to compute minimum detectable change in density for power ≥ 0.80.

Table 2. Species found in excavated quadrats at 14 sites that were sampled June-September 1997.

Species	Sites	Count
Actinonaias ligamentina	Allegheny, French Creek	118
Alasmidonta heterodon	Ashuelot, Connecticut, Neversink	65
Alasmidonta marginata	Allegheny, French Creek	14
Alasmidonta undulata	Ashuelot, Connecticut, Farmington, St. George, West	62
Alasmidonta varicosa	Cacapon, Neversink, Piscataquog, St. George, West	31
Alasmidonta viridis	Little Tennessee	1
Amblema plicata	French Creek	3
Anodonta implicata	Neversink	1
Elliptio complanata [a]	Cacapon, Connecticut, Neversink, Norwich Creek, Piscataquog, Poultney, St. George, West	510
Elliptio dilatata	Allegheny, French Creek, Little Tennessee	97
Elliptio fisheriana	Norwich Creek	20
Epioblasma torulosa rangiana	Allegheny, French Creek	12
Fusconaia subrotunda	French Creek	1
Lampsilis cardium	French Creek	1
Lampsilis cariosa	Cacapon, St. George	2
Lampsilis fasciola	Allegheny, French Creek, Little Tennessee	7
Lampsilis ovata	Allegheny, Poultney	5
Lampsilis radiata	Poultney	14
Lasmigona costata	Allegheny, French Creek, Poultney	12
Lasmigona subviridis	Little	20
Leptodea fragilis	Poultney	6
Ligumia recta	Allegheny	3
Pleurobema clava	Allegheny	2
Pleurobema sintoxia	French Creek	1
Potamilus alatus	Poultney	4
Ptychobranchus fasciolaris	French Creek	1
Pyganodon cataracta	Ashuelot, Farmington	3
Pyganodon grandis	Poultney	1
Quadrula cylindrica	French Creek	1
Strophitus undulatus	Ashuelot, Farmington, Neversink, Poultney, West	18
Villosa fabalis	Allegheny, French Creek	109

[a] *E. complanata* was found, but not counted at Ashuelot and West Branch of the Farmington Rivers.

Results

Factors affecting detection: a case study

We found 31 species in excavated quadrats (Table 2). The most widespread was *E. complanata,* which we found at all of the Atlantic Slope sites (however, counts of *E. complanata* were not recorded at Ashuelot or West Branch of the Farmington Rivers due to its very high abundance). Because we wanted to examine detection across a wide range of conditions, we focused the analysis of detection and habitat on *E. complanata*.

Preliminary analyses led us to drop some of the explanatory variables prior to performing logistic regression analysis. We excluded temperature in the model because our surveys occurred during summer months and temperature varied little (*i.e.*, 16-23 °C). We dropped turbidity because of its relationship to substrate size. Fine sediments such as organic debris and silt were found only where turbidity was high (≥6 NTU); and coarse material such as gravel, cobble, and boulder was found only where turbidity was medium or low (<6 NTU). Macrohabitat was dropped because it was related to depth. (Riffles were limited to depths < 0.65 m; pools and runs were found in a wide range of depths.) To represent habitat in the model we retained depth, percent vegetation, and substrate size.

We found significant observer effects within 3 of the 8 sites where *E. complanata* was recorded. However, when comparisons among observers were made

conditional on habitat, observer effects were limited to 1 or 2 substrate types per site. At the Cacapon River site there was an observer effect among 4 observers in sand (χ^2 =12.34, df=2, P=0.002) and small cobble (χ^2 = 7.72, df=3, P=0.043), but not in large cobble (χ^2 =3.18, df=3, *p* =0.52) and boulder (χ^2=4.13, df=3, P=0.29). At the Little River site there was an observer effect among 6 observers in silt (χ^2=40.3, df=5, P<0.001), but not in organic debris (χ^2=5.7, df=4, P=0.24), clay (χ^2 =0.15, df=2, P=1.00), or sand (χ^2 =3.7, df=5, P=0.62). At the Neversink River site there was an observer effect among 6 observers in large cobble (χ^2=14.9, df=5, P=0.063), but not in small cobble (χ^2 =3.8, df=5, P=0.70). We did not want to diminish the importance of observer effects, but wanted to examine effects of habitat and mussel length on detectability. Thus, we chose to pool data across observers and concentrate on modeling detection as a function of habitat and length of the mussel. We feel confident that this approach did not compromise analysis because regression diagnostics showed that lack of fit in the best fitting model was not related to observers.

We found that the probability of detection was significantly related to depth, percent vegetation, substrate size, and mussel length (χ^2 =174.3, df=25, P< 0.0001). The best fitting model included complex interactions between the explanatory variables. The effect of mussel length appeared to be strongest in silt and sand (Fig. 1), but weak in gravel where the effect depended on percent rooted vegetation. We found no length effect in small cobble (χ^2=0.259, df=1, P= 0.611). In 4 of the 8 substrate types, the greater the percent of rooted vegetation the lower the probability of detection (Fig. 1). However, in gravel and small cobble the data suggests the opposite effect–the greater percent vegetation the more likely that a mussel would be detected. We found no apparent relationship between vegetation and detection in substrates of sand or organic debris. Consistently among substrates, there was a depth effect characterized by slightly higher detection at intermediate depths (0.25-0.75 m) than in shallow (<0.25 m) or deeper (0.75-1.5 m) water (Fig. 1). The lowest detection was found in deeper water (0.75-1.5 m). Differences in

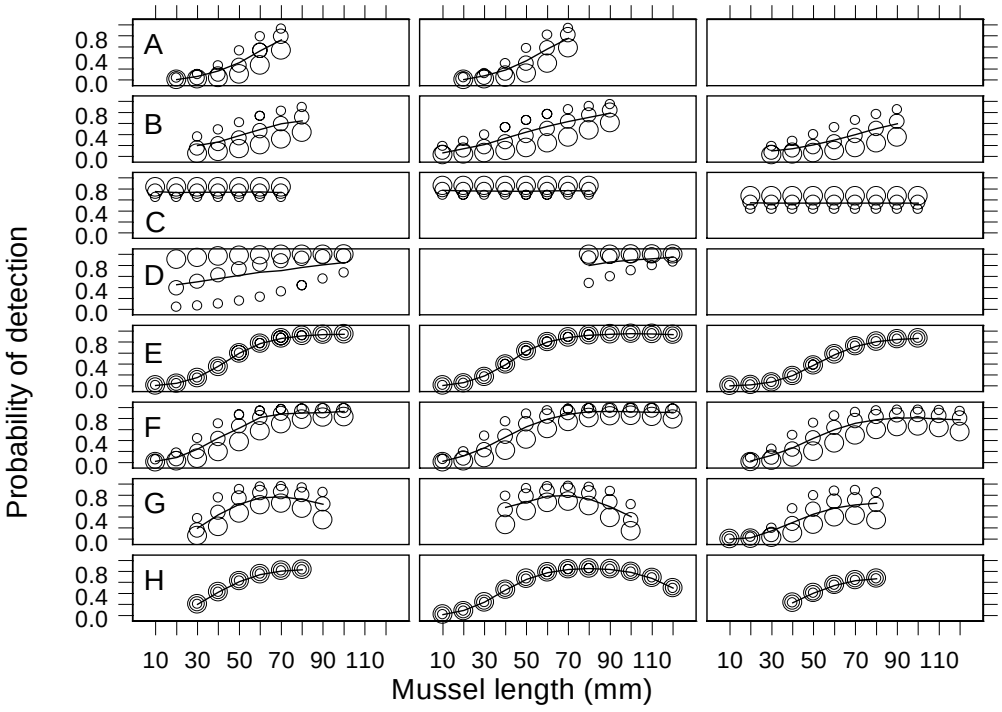

Figure 1. Probability of detection as a function of mussel length (measured along its longest axis) and habitat. Habitats are defined by substrate type (boulder: row A, large cobble: row B, small cobble: row C, gravel: row D, sand: row E, silt: row F, clay: row G, and organic debris: row H), depth (<0.25 m: left column, 0.25 to 0.75 m: middle column, and 0.75 to 1.5 m: right column), and percent rooted vegetation (0: small circle, 0 to 33: medium circle, and >33: large circle). Probability of detection is the predicted probability that an *E. complanata* is detected during a search of the substrate surface. The probability of detection is based on a logistic regression model of data from 8 Atlantic Slope streams where 521 0.25 m^2 were excavated to a depth of approximately 10 cm after a visual or tactile search of the surface.

Table 3. Relative abundance based on surface and total counts for 2 sites where abundance rankings differed between surface and excavated samples. Total counts are mussels on the surface plus mussels detected by excavating to a depth of approximately 10 cm.

Site	Species	Total Count %	Total Count Rank	Surface Count %	Surface Count Rank	% Detected at the Surface
Ashuelot River [a]	*Alasmidonta heterodon*	36.7	1	20.0	3	22
	Alasmidonta undulata	34.7	2	35.0	2	41
	Strophitus undulatus	26.5	3	40.0	1	62
	Pyganodon cataracta	2.1	4	5.0	4	100
French Creek	*Villosa fabalis*	41.5	1	23.3	2	25
	Actinonaias ligamentina	34.1	2	57.8	1	71
	Elliptio dilatata	7.9	3	1.4	7	8
	Alasmidonta marginata	6.7	4	5.5	3	36
	Lasmigona costata	3.0	5	4.1	4	38
	Amblema plicata	1.8	6	4.1	4	100
	Epioblasma torulosa rangiana	1.2	7	2.7	6	100
	Fusconaia subrotunda	0.6	8	1.4	7	100
	Lampsilis cardium	0.6	8	1.4	7	100
	Quadrula cylindrica	0.6	8	1.4	7	100
	Lampsilis fasciola	0.6	8	0	-	0
	Pleurobema sintoxia	0.6	8	0	-	0
	Ptychobranchus fasciolaris	0.6	8	0	-	0

[a] Relative abundance of species other than *Elliptio complanata* are shown for the Ashuelot River. *E. complanata* was the most abundant species at the Ashuelot River; however, its numbers were not recorded.

detection among substrate types were greatest for younger (and smaller) mussels (Fig. 1).

Some of the patterns that emerged from modeling detection could be spurious although patterns were largely consistent with our perception of how detection changed with habitat and mussel length. For example, the model indicated that in gravel and small cobble the probability of detection increased with rooted vegetation. At first this result seemed counterintuitive, however we offer the following heuristic argument that the result may be accurate. We suggest that rooted vegetation increases the proportion of mussels at the surface similarly for all substrates, however "visibility" of mussels at the surface varies among substrates. Increased root mass will occupy space that otherwise would be available for mussels. Thus, in a sense, mussels are forced to the surface by the increase in rooted vegetation regardless of substrate. However, the effect on detection of mussels at the surface might differ among substrates. There are three cases to consider: fine sediment (*e.g.*, silt and clay), intermediate sediment (*e.g.*, gravel and small cobble), and coarse sediment (*e.g.*, large cobble and boulder). In fine sediment, presence of vegetation hinders visibility of mussels at the surface because turbidity will increase when vegetation is parted to search for mussels and because vegetation interferes with tactile searching. Increased turbidity might be less of a problem in the other two substrates. In coarse sediment, mussels are hidden amongst larger material, and the presence of vegetation compounds that problem. In intermediate sediment, there is less turbidity than in fine sediments and less surface roughness than in coarse sediments. Thus, we hypothesize that in intermediate sediment as rooted vegetation increases more mussels are at the surface, yet visibility is not greatly reduced, and the net effect is higher detection.

Another interesting result was that detection was greatest at intermediate depths. We offer a possible explanation. At shallow depths the observer's field of view is restricted because his/her face is close to the substrate. Consequently, coverage is compromised. At intermediate depths the observer can "pull back" and enjoy a wider and possibly more effective field of view of the substrate in the quadrat. As depth increases the effect of turbidity increases, thus decreasing visibility.

At 12 (86%) of the 14 sites relative abundance as measured by ranks from surface counts matched that from total counts. However, at 2 (14%) of the sites (Ashuelot River [P=0.08] and French Creek [P= 0.06]) the ranking of the most abundant species changed order

Table 4. A comparison of densities among sites for two Federally endangered species (*Alasmidonta heterodon* and *Epioblasma torulosa rangiana*) as calculated from surface and total counts.

Species	Site	Total Density (no. m^{-2}) SE	Surface Density (no. m^{-2}) SE	% Detected at the Surface
A. heterodon	Ashuelot River	1.11 ± 0.424	0.25 ± 0.148	22
	Connecticut River	2.45 ± 0.592	1.31 ± 0.456	55
	Neversink River	0.71 ± 0.216	0.45 ± 0.188	64
E. t. rangiana	Allegheny River	0.31 ± 0.098	0.20 ± 0.081	67
	French Creek	0.29 ± 0.286	0.29 ± 0.286	100

when based on surface counts rather than total counts (Table 3). For example, at the Ashuelot River site *Alasmidonta heterodon* (Lea, 1830), a federally listed species, was ranked 1st and *Strophitus undulatus* (Say, 1817) ranked 3rd based on total counts (not including *E. complanata*). However, when based on surface counts *A. heterodon* ranked 3rd and *S. undulatus* ranked 1st because 22% of *A. heterodon* and 62% of *S. undulatus* were detected at the surface (Table 3).

Because percent detected at the substrate surface varied among sites, comparisons of species status among sites depended on whether density was calculated from total or surface counts (Table 4). Based on surface counts, the Ashuelot River site appeared to have the lowest density (0.25 m^{-2}) of *A. heterodon* (Table 4). However based on total counts, *A. heterdon* at the Ashuelot River site was 1.11 m^{-2}, an intermediate density compared to the other two sites where we found the species. Similarly, surface density of *Epioblasma torulosa rangiana* (Lea 1838) was 45% greater at the French Creek site than at the Allegheny River site, but total density was comparable between the 2 sites (Table 4).

We observed mortality in 2 of the 12 species held for 7 weeks (Table 5): *Villosa fabalis* (Lea 1831) and *Alasmidonta undulata* (Say 1817). In neither case was mortality related to excavation (for *V. fabalis*: χ^2=0.402, df=1, exact P=0.643; and for *A. undulata*: χ^2=2.27, df=1, exact P=0.259). Overall, mortality was 11.7% (8 of 68) for *A. undulata* over 67 d and 6.7% (4 of 60) for *V. fabilis* over 50 d.

The optimal proportion to excavate

Excavation was 3 to 12x more time consuming than surface counts. Typically, excavation took 6x longer than surface counts; at 75% of the sites excavation took > 4x longer. Strength of the relationship between total counts and surface counts varied among sites and depended on detectability of mussels at the site (Table 6).

The optimal proportion to excavate (*i.e.*, the proportion that minimized variance for fixed cost as determined from Eqn. 1) ranged from about 10% to 100% and was related to the percent detected at the surface (Fig. 2; on log scale r = -0.94, t =-9.24, df=12, P< 0.0001), but was not related to density (on log scale r = 0.07, t = 0.24, df=12, P=0.81). Although we used Eqn. 1 and Eqn. 2 to calculate the optimal proportion to excavate using observations from our 14 sites, we used Eqn. 3 and Fig. 3 to illustrate graphically the numerical procedure. For example, to use Fig. 3 to find the proportion that minimizes the variance (*i.e.*, to find the optimal proportion of quadrates to excavate), follow 1 of the variance curves to its lowest point then drop down to the *x*-axis. This is in effect what was done by the use of Eqn. 1, although Eqn. 1 provides an exact numerical result. The minima depended on the strength of the relationship between surface and total counts (*i.e.*, $R^2_{adjusted}$), which in turn is determined by the percent detected at the substrate surface (*i.e.*, the higher the percent the stronger the relationship). By examining the variance curves, we noticed that the variance curve flattened around the minimum as $R^2_{adjusted}$ decreased (Fig. 3). Thus, as $R^2_{adjusted}$ decreased a wider range of the proportion to excavate came close to minimizing the variance.

We summarized results on how much to excavate to yield robust estimates of population density given percent detection at the substrate surface (Table 7). We reduced the results to 4 possible cases. If >60% were detected at the substrate surface then variance was approximately minimized by excavation of 25% (or 1 out of 4) of the quadrats. Similarly, 50 to 60% detection at the surface resulted in 33% excavation, 40 to 50% detection at the surface resulted in 50% excavation, and <40% detection at the surface resulted in 100% excavation.

Coefficient of variation was a function of density and sample size; the higher the density and sample size, the lower the CV (Fig. 4). For sample size = 200, CVs were 0.25, 0.33, 0.45, and 0.69 for densities of 1.0,

Table 5. Evaluation of the survivorship of mussels removed by excavation and held in cages to determine if excavation increased mortality. Mortality was observed for 2 species: *Villosa fabalis* at French Creek and *Alasmidonta undulata* at Piscataquog River. In neither case was mortality attributable to excavation.

			Excavated			Surface		
Site	**Days Held**	**Species**	**Count**	**Mean Length (SD)**	**% Dead**	**Count**	**Mean Length (SD)**	**% Dead**
Cacapon River	119	*Elliptio complanata*	42	46.3 (9.7)	0	31	59.6 (13.1)	0
French Creek	50	*Actinonaias ligamentina*	8	42.3 (23.1)	0	5	69.2 (16.2)	0
		Alasmidonta marginata	4	69.1 (12.9)	0	12	59.4 (10.9)	0
		Amblema plicata				1	82.2	0
		Elliptio dilatata	8	71.2 (23.7)	0	8	65.4 (20.1)	0
		Lasmigona costata	2	87.8 (39.9)	0	6	99.0 (16.7)	0
		Lampsilis fasciola	1	35.1	0			
		Pleurobema sintoxia	1	60.5	0			
		Ptychobranchus fasciolaris	1	52.1	0	1	102.2	0
		Strophitus undulatus				1	61.8	0
		V. fabalis	36	23.5 (5.2)	8.3	24	26.2 (5.5)	4.2
Piscataquog River	67	*A. undulata*	34	38.9 (8.5)	5.9	34	44.1 (7.6)	17.6
		Elliptio complanata	35	56.2 (17.7)	0	51	61.0 (15.1)	0

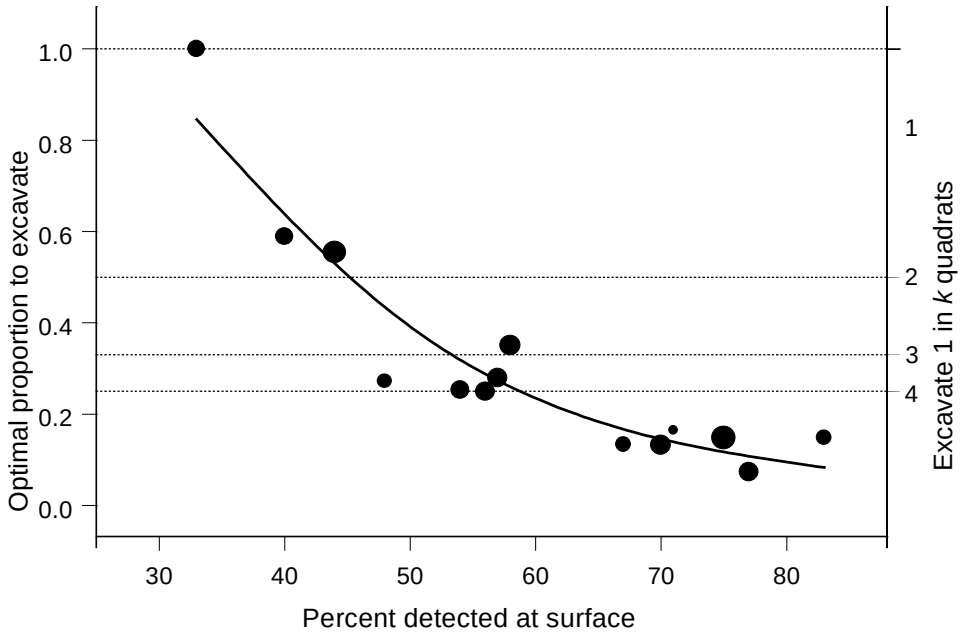

Figure 2. Optimal proportion excavated as a function of percent detected at the substrate surface based on mussel surveys at 14 sites. The proportion excavated is optimal in the sense that it yields a population estimate with minimum variance for a fixed cost. The size of the symbols is proportional to mussel density at each site. On the 2nd y-axis is the interval between excavated quadrats when those quadrats are selected systematically.

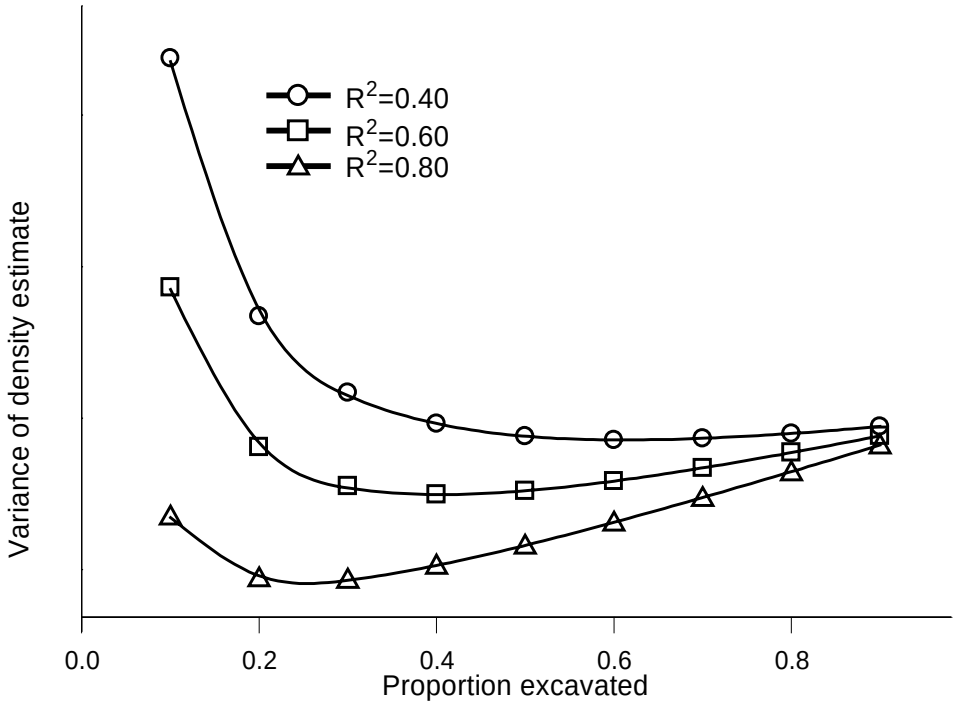

Figure 3. Variance of a density estimate as a function of the proportion excavated. The proportion excavated refers to the proportion of an initial sample of quadrats that is excavated in a double sampling design. On the initial sample, only a surface count is conducted. Variance is based on a regression estimator. The shape of the variance curve is related to the strength of the relationship between surface counts and total counts as measured by R^2.

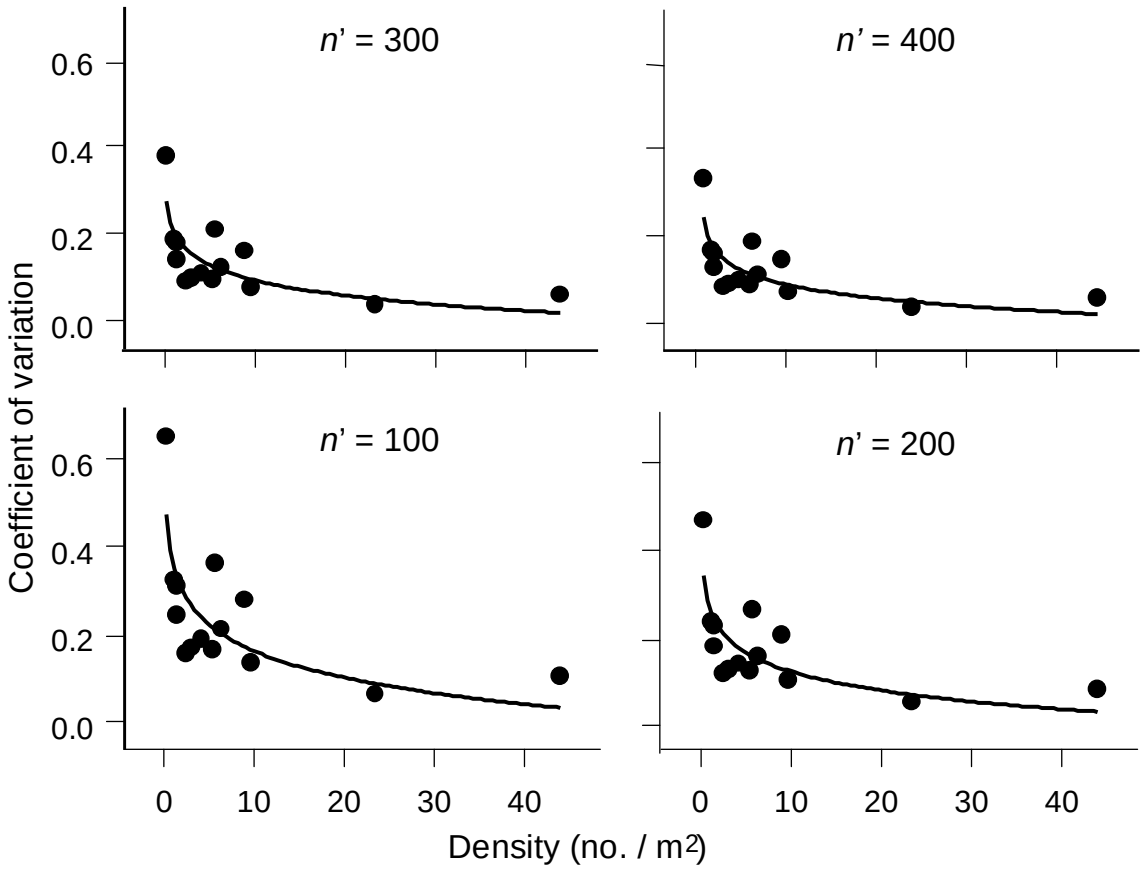

Figure 4. Coefficient of variation calculated for a range of sample sizes and based on a double sampling design where only a proportion of sampled quadrats are excavated. Calculations were based on data from the 14 sites.

0.50, 0.25, and 0.10, respectively. For sample size = 400, CVs were 0.18, 0.23, 0.32, and 0.49 for densities of 1.0, 0.50, 0.25, and 0.10, respectively. When density was transformed to an inverse square root scale, CV was a linear function of density (t = 6.06, df=12, P< 0.0001); and this relationship was used to generalize the power analysis.

The minimum detectable change in density over 5 or 10 y, like variance, depended on density and sample size (Fig. 5). Also, the more years of monitoring the smaller the density change that would be detected. Sample size had a qualitatively more dramatic effect on detecting increases in density. When the focus was on detecting drops in density below a certain level, say below 0.10 m^{-2} for example, detection of change depended on initial density and sample size. To detect a drop below 0.10 m^{-2} for 5 y of monitoring, initial density needed to be ≥0.6 m^{-2} with sample size ≥200. In contrast, to detect a drop below 0.10 m^{-2} for 10 y of monitoring, initial density could be as low as 0.2 m^{-2} with sample size ≥400; that would be equivalent to detecting a drop of 100 individuals per 1000 m^2.

Discussion

Detection at the substrate surface was related to observer, habitat, mussel length, and mussel species. Thus, changes in any of these variables will confound comparison of populations across time, sites, habitat, or taxa when based exclusively on surface counts. For example, in the absence of rooted vegetation the probability of detecting a 40 mm *E. complanata* was 74% higher in small cobble (probability = 0.68) than in sand (probability = 0.39), and comparing surface densities across these microhabitats would lead to erroneous conclusions about habitat use. At a larger scale, we found that surface density was an unreliable indicator of population status because detection varied among sites. For example, detectability of *A. heterodon* at the Neversink site (0.64) was 191% of that at the Ashuelot site (0.22) so that densities (based on surface counts) appeared higher at the Neversink site when, in fact, the opposite was true. Because detection was species-specific, comparison of relative abundance based on surface counts within the same site can be misleading. Even ranked

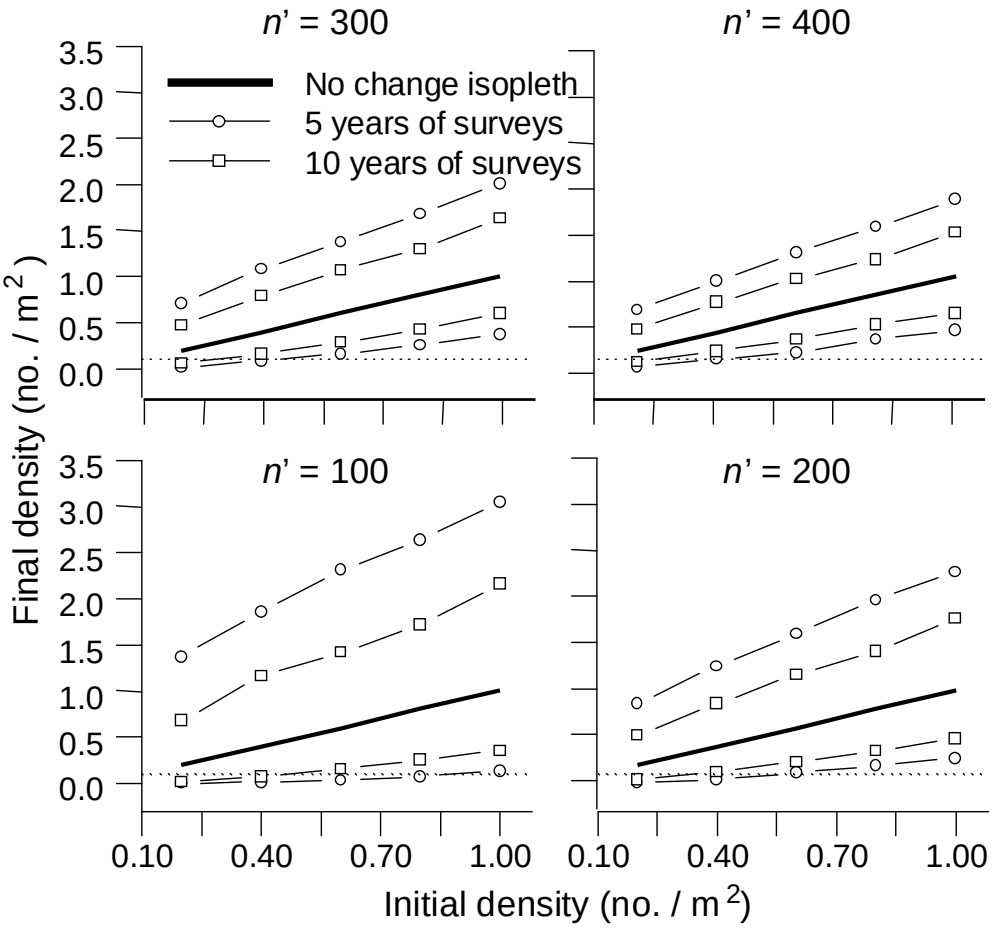

Figure 5. Minimum detectable changes in density for monitoring over 5 or 10 y and for various levels of sample size. The change in density is the smallest that would be detected with 80% probability given a 10% Type I error rate, a one-tailed test, and assuming a trend that is proportional to density. The underlying sampling design is double sampling with an optimal proportion of the sample being excavated.

abundance based on surface counts was an inaccurate measure of relative abundance at 2 (14%) of the 14 sites.

One strategy to cope with variable detection in "qualitative" or "semi-quantitative" sampling (Miller and Payne 1988) is to standardize survey methods and hold constant the conditions under which surveys are conducted. This strategy will not be successful because it is not possible to hold constant habitat variables such as depth, vegetation, and substrate. Biomass of submerged aquatic vegetation varies temporally and spatially, substrate is altered by fluvial processes, and flow may not drop to a base level in years with higher than average precipitation. Thus, critical microhabitat conditions vary temporally and spatially in spite of the intentions of the surveying biologist.

Our results are consistent with earlier work regarding vertical position of *E. complanata.* Amyot and Downing (1991) and Balfour and Smock (1995) found variation across season and mussel length. Because we found that probability of detection was lowest and differences in detection across all microhabitats were greatest for small mussels, our results underscore the recommendations of Miller and Payne (1988), Richardson and Yokley (1996), and Vaughn *et al*. (1997) that recruitment will be more difficult to observe without including excavation in the survey protocol.

We conclude that when the objective is to rigorously compare population density across sites, time, habitat, or taxa, it is not a question of whether to excavate, but rather how much to excavate. However, the benefit of excavation in terms of increased accuracy must be weighed against the added cost due to increased effort required.

Table 6. Results from linear regression of total count as a function of surface count. The , which is defined in the text, can be interpreted as conventional R^2. Percent detected at the surface is a ratio of the surface count over total count. Density (no. m^{-2}) is estimated from total counts.

Site	Regression Parameters $(\hat{\beta}_0, \hat{\beta}_1)$	$R^2_{adjusted}$	% Detected at the Surface	Density (no. m^{-2}) ± SE
Ashuelot River	(0.53, 1.07)	0.31	41	3.02 ± 0.519
Cacapon River	(0.42, 1.27)	0.78	58	6.36 ± 1.369
Connecticut River	(0.38, 1.16)	0.69	55	4.16 ± 0.808
Little River	(2.37, 1.04)	0.93	75	44.00 ± 6.050
Neversink River	(1.01, 1.00)	0.65	58	9.68 ± 1.280
Norwich Creek	(0.51, 1.07)	0.92	71	8.92 ± 3.159
Piscataquog River	(0.02, 0.99)	0.81	71	0.28 ± 0.130
St. George River	(0.12, 1.16)	0.53	48	1.08 ± 0.236
West River	(0.02, 1.39)	0.87	68	1.44 ± 0.396
Farmington River, W. Branch	(0.04, 1.07)	0.87	83	1.44 ± 0.375
Allegheny River	(0.53, 1.07)	0.74	57	5.39 ± 0.611
French Creek	(3.27, 0.99)	0.47	44	23.44 ± 2.352
Little Tennessee River	(0.42, 0.98)	0.14	33	2.52 ± 0.792
Poultney River	(0.19, 1.11)	0.94	78	5.72 ± 1.538

We make recommendations on how much to excavate that are based on the double sampling design and a minimizing of variance for fixed survey cost. We recommend that under the double sampling design the proportion of quadrats excavated should be determined by the percent of mussels likely to be detected at the substrate surface. We summarized results into a simple set of rules for determining how much to excavate (Table 7). To use this set of rules, information on the percent likely to be detected at the substrate surface for the species of interest is needed. These preliminary data may exist from similar surveys or may be obtained through a pilot survey.

Table 7. Recommended rules for determining the optimal proportion of quadrats to excavate. Percent detection at the surface refers to the percent of the species that is likely to be detected at the substrate surface, which could come from a pilot survey or similar surveys. A convenient and valid method to select a subset of quadrats for excavation is by excavating every k^{th} quadrat (or excavate "1-out-of-*k*" quadrats). In other words, which quadrat to excavate can be determined systematically with the first chosen at random among the first *k* quadrats.

% detection at the surface	Optimal proportion to excavate	*k*
> 60%	0.25	4
50-60%	0.33	3
40-50%	0.50	2
<40%	1.00	1

In our calculation of the optimal proportion to excavate, we formulated survey costs simply in units of time. However, the true cost of excavation includes disturbance to mussels and their habitat. If quantified, then disturbance can be incorporated in the analysis to find the optimal amount of excavation. If disturbance were much greater in excavation than in surface counts, then the optimal proportion to excavate would be lower than what we report here. However, we found no evidence that excavation increased mortality. We did observe mortality in 2 of the 12 species held after sampling; 11.7% of *A. undulata* over 67 d and 6.7% of *V. fabalis* over 50 d. If not due to excavation per se, then the mortality was likely related to the action of removal from and re-embedding to substrate, which occurs during most survey techniques. Nevertheless, we conclude that significant effects of sampling on population-level survival are unlikely because a small percentage of a site is sampled in a typical survey. Thus, most of the sampled mussels will not be affected adversely. For example, if 10% of a site is sampled (*i.e.*, 400 0.25 m^2 quadrats in a 1000 m^2 site) and sampling causes 20% mortality of sampled mussels (this level of mortality was higher than we observed, cf. Table 5), then sampling would cause only 2% mortality for the population. This would be a worse than expected scenario because more often than not we observed no mortality among

sampled mussels and typically <10% of a site is sampled (Table 5).

The next step, after finding the optimal proportion to excavate, is to calculate sample size. Under the double sampling design, sample size is the total number of quadrats on which to conduct a surface count (*i.e.*, n'); of these, a proportion is excavated. Sample size is primarily a function of density. Sample size needed to achieve precise density estimates for densities ≥1.0 m^{-2} is in the order of 100-200 0.25 m^2 quadrats. If, for example, 40-50% of the mussels are likely to be detected on the surface, then 50% of the quadrats should be excavated. The time required to conduct the survey would be 6.7-13.3 h (not including time to set up and break down), assuming an average of one minute for a surface count and six minutes for an excavation. The actual time to complete the survey will depend on the crew size. In our experience, such a survey would take 1-2 d with a crew of five or six. However, to achieve precise density estimates for densities <1 m^{-2} will require 2-4 times the effort required to effectively sample densities ≥1.0 m^{-2}. Double sampling can be combined with more complex designs useful for sampling rare populations, such as stratification based on density and adaptive cluster sampling (Thompson 1992, Strayer *et al*. 1996).

We disagree with Payne *et al*. (1997) who suggest it is not worthwhile to estimate density of low-density populations (*i.e.*, those <0.5 m^{-2}). First, Payne *et al*. (1997) do not consider possible gains in efficiency due to improved survey design. We calculate that use of the double sampling design would result in a CV = 0.23 for a population of 0.5 m^{-2} and a sample size of 400. This is less than half the sample size that Payne *et al*. (1997) predict would be needed for a similar CV and population density; the difference is due to improved survey design. Second, in survey planning there is an overemphasis on CV to determine adequacy of sample size. CV measures variance relative to the magnitude of the density estimate; as density decreases it takes a smaller absolute variance to achieve a low CV. Whereas relative variance (as measured by CV) is very useful for survey planning, absolute variance and power analysis should also play a role. For example, at a site where density is 0.2 m^{-2}, sample size of 400 would result in an estimate with CV of 0.36, a SE of 0.07, a "margin of error" (2 SE) of 0.14 or 140 individuals per 1000 m^2 site, and high power (>80%) to detect drops to 0.02 m^{-2} over 5 y or 0.07 m^{-2} over 10 y. Although the CV of 0.36 does not meet the criteria of CV=0.20 suggested by Payne *et al*. (1997:154), we argue that this estimate is informative and when applied to a monitoring program meaningful changes in population density would likely be detected.

To document presence of rare species it is clear that some amount of directed qualitative sampling is required (Strayer *et al*. 1997, Vaughn *et al*. 1997). However, we offer the following reasons for quantitative sampling even for rare populations (*i.e.*, densities ≤0.1 m^{-2}). First, in the absence of quantitative sampling (used here to be analogous to probability sampling) there is no measure of uncertainty and therefore no way to gauge the reliability of the survey results. Second, even though target species may occur at low density, often other species at a site occur at higher density and can be used to monitor changes in the mussel community. Third, if quantitative sampling is used to monitor a population, then population recovery can be documented. Fourth, quantitative sampling allows a probability statement to be made regarding species presence and maximum density. For example, to model the probability of detecting rare species Green and Young (1993) used the Poisson distribution. That same approach can be adapted to make statements regarding species presence and maximum density when only a proportion of quadrats are excavated. For our double sampling design the equivalent formulae to Eqn. 3 in Green and Young (1993) is

$$n' = \frac{-4\ln(\beta)}{m\left(\left[1-f_2\right]\lambda + f_2\right)}$$

where m is density (no. m^{-2}), n' is number of quadrats in the sample, f_2 is proportion of n' that is excavated, λ is the proportion of mussels detected at the substrate surface, and β is the probability of not detecting any mussels in the n' quadrats. The multiple '4' is needed because mussel density is expressed in numbers m^{-2}, whereas quadrat area is 0.25 m^2. Take, for example, the following scenario: percent detected at the surface was 60% (λ=0.60) based on excavation of 33% of the quadrats (f_2=0.33) and suppose 200 quadrats are sampled (n'=200). In this scenario, if the target species of mussel was not found then with 95% confidence (β=0.05) we can state that species density at the site was ≤0.08 m^{-2}. The time to sample the 200 quadrats, 67 of which are excavated, would require approximately 10 h of search time (using 1 min for surface counts and 6 min to excavate). Search time can be divided among several observers so that field time would be less than 10 h.

We agree with Thompson *et al*. (1998) who state, "sampling rare populations will likely be a very costly

endeavor regardless of how it is performed". We offer suggestions on sampling techniques that provide some gains in efficiency. However, we ultimately conclude that to successfully monitor populations of mussels, particularly those that occur at densities <0.4 m^{-2} requires a substantial investment to collect the necessary data. If monitoring population density is an objective given high priority then managers must be prepared to allocate adequate time and money to the task.

Acknowledgements

Funding was provided by USGS Biological Resources Division through the Species at Risk Program and U.S. Fish and Wildlife Service, Silvio O. Conte National Fish and Wildlife Refuge. We are indebted to the following individuals for their participation in data collection: John Alderman, J. Allen, Bob Anderson, J. Baldizar, Henry Barbour, Bill Bartles, Glen Black, Marc Blouin, D. Braden, H. Brooks, D. Brown, Janet Butler, Janet Clayton, Tom Cox, Mark Ferguson, Chris Fichtel, Steve Fiske, T. Flynn, John Fridell, Marea Gabriel, M. Gibson, Beth Goettel, T. Gola, Marnee Gormley, Tom Gloria, Jamie Haskins, Carrie Horton, Pat Huckery, Judith Johnson, John Kanter, Bill Lellis, Madeleine Lyttle, H. Malcom, Paul Marangelo, Mark Maurer, David McLain, Mark McCollough, Debbie Mignogno, Robert Monroe, Patty Morrison, Tom Muir, Kathleen O'Brien, Amy Pasternak, Mike Penko, Lois Richards, Kathy Schneider, David Strayer, Rebecca Suomala, Nissa Thomsen, Keith Voges, J. Weitzel, Dave Weller, Barry Wicklow, M. Wood, and Priscilla Young. We offer a special thanks to Paul Marangelo, Nissa Thomsen, and Carrie Horton for their coordination of field work. We thank Penelope Pooler, Paul Johnson, and 2 anonymous reviewers for helpful comments on an earlier draft of the manuscript.

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Appendix

Formulae for estimating density using the regression estimator under the double sampling design are (Thompson 1992, Hedayat and Sinha 1991)

$$\hat{\mu}_{lr} = a^{-1}\left[\bar{y}_2 - \hat{\beta}_1\left(\bar{x}_2 - \bar{x}_1\right)\right], \qquad \textbf{(A.1)}$$

with variance

$$\operatorname{var}\left(\hat{\mu}_{lr}\right) = a^{-2}\left\{\left(\frac{N-n'}{N}\right)\frac{s^2}{n'} + \left[\frac{n'-n}{n'n(n-2)}\right]\sum_{i=1}^{n}\left(y_i - \hat{\beta}_0 - \hat{\beta}_1 x_i\right)^2\right\}, \qquad \textbf{(A.2)}$$

where a is the quadrat area, is the mean total count from second-phase sample, and are the mean surface counts from the first and second-phase samples, and are estimates of the regression parameters (*i.e.*, intercept and slope), is the variance of total counts in the second-phase sample, N is the total number of quadrats at a site (*i.e.*, total site area/quadrat area), n' is the size of the first-phase sample, and n is the size of the second-phase sample.